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Effect of *Paramuricea clavata* (Risso, 1826) "forest" on the benthic populations of Tremiti Islands

"Effetto foresta" di *Paramuricea clavata* (Risso, 1826) su popolamenti bentonici delle Isole Tremiti

Tesi di laurea in Habitat Marini: rischi e tutela

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I'm forever walking upon these shores,
Betwixt the sand and the foam.
The high tide erase my foot prints,
And the wind blow away the foam.
But the sea and the shore remain
Forever.
(Kahlil Gibran, 1990)

Sempre camminerò per queste spiagge,
Tra la sabbia e la schiuma dell'onda.
L'alta marea cancellerà l'impronta
E al vento svanirà la schiuma.
Ma spiaggia e mare rimarranno
Per sempre

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1 Introduction

1.1 The Mediterranean coralligenous assemblages

Mediterranean coralligenous habitats are temperate biogenic reefs mainly produced by the accumulation of calcareous encrusting algae, growing in dim light conditions (Ballesteros 2006). These habitats can be found from 20 m to 120m in depth. The concretions can develop on vertical or horizontal surfaces, and species assemblages are largely affected by the irradiation intensity. Coralligenous habitats host high species diversity, comparable to those of *Posidonia oceanica* meadows (Boudouresque 2004). In this environment there are several species belonging to different taxonomic groups like sponges, molluscs, bryozoans, tunicates, crustaceans and fishes. Coralligenous habitats offer protection to several vertebrates and invertebrates from their predators.

In the years the estimates of species that residing in the coralligenous assemblages was updated but the real coralligenous species composition is not still well known. There are several problems related at the difficult access at the sampling sites, the high diversity and the complexity of the substrata limit the researches (Kipson et al. 2011). The base-line level of biodiversity studied in the past decades is well shown by Ballesteros (2006), on his review the number of species in the coralligenous assemblages was estimated in 142. A recent review has increased this estimation to272 species (Bertolino et al. 2013). Besides to biodiversity, the beauty of these habitats attract recreational divers (Harmelin 1993).

The morphologies of coralligenous structures can be distinguished in banks or rims. The first is a plane frame with a range of thickness from 0.5 to some meters. This type of structures can present ravines with numerous holes. It is easy to find this type of morphology on rocky substrata, where coralligenous covers the rocky outcrops. The second type of coralligenous structures growth on vertical rocky substrates or on the entrance of caves.

1.1.1 Environmental variable affecting coralligenous assemblages

The distribution of benthic organisms on rocky substrata in the Mediterranean Sea is largely affected by light conditions (Ballesteros 1992, Irving and Connel 2002, Martí et al. 2004, 2005). Algae, the main builders in the coralligenous framework, needs light to growth but at low level of irradiance (Pérès and Picard 1964, Laubier 1966). On the substrata the irradiances should be between 0.05% to 3% of the surface values (Ballesteros 1992) and in the hole and in the crevices of coralligenous banks maybe almost zero like in the innermost of cave or in the bathyal zone. Blue and green wavelengths is the only that arrive at the surface of coralligenous substrate (Ballesteros 1992). These wavelengths are suitable for red algae that are the most dominant algae in this community (Ballesteros 1992).

Coralligenous assemblages can be found in a wide range of water trophic conditions. Neverless, high nutrients concentrations can inhibit the coralligenous construction and increase the destruction rates (Hong1980).

Substrate orientation and inclination play a relevant role in structuring the coralligenous assemblages (Carleton and Sammarco 1987, Baynes 1999, Glasby 2000, Glasby and Connel 2001, Virgilio et al. 2006). Temperature is a primary factor on distribution of coralligenous species. Changing of Mediterranean climate may cause mass mortality events of benthic organisms. Geographically the Mediterranean Sea is located in temperate area with four distinct seasons, characterized by warm summer, quite cold winter and mild springs and autumns with high rain events. The seawater temperature is influenced by season, but the response of water is slower than changing of temperature in atmosphere. In the recent years there has been a different thermal anomalies, wave of heat at the end of the summer, that cause an increase of temperature of atmosphere for long periods. Like the atmosphere, the seawater become warm. These events, generally occurring during low hydro-dynamism condition in late summer, act as stress factor for coralligenous animals and seaweeds. In recent years there were some episodes documented of heat that have produced mass mortality events for five invertebrate phyla (Cordata (Tunicata), Bryozoa, Cnidaria, Mollusca and Porifera; Bally et al.2007). Some organisms in coralligenous assemblage present a stenothermal behaviour and are sensible to changing of usual value of temperature. Moreover, the increase of temperature can cause the growth of bacterial strains that can act like a stressor agents for debilitated organisms.

Another example is the large-scale mortality of benthic suspension feeders in coralligenous assemblage caused by the high temperature summer periods (Romano et al. 2000, Garrabou et al. 2009). The impact of this mortality could be high, because if there are a periods of hot water, year by year, we could observe the decline of these species (Cerrano et al. 2000).

1.1.2 Biological interactions

The coralligenous assemblage (Figure 1) can support several forms of life that utilize this habitat as protection, substratum to growth and uptake nutriment.

Organisms composing the coralligenous assemblages can be distinguish in (Hong 1982,1980): 1) fauna contributing to build the structures by their calcareous body and/or agglomerating carbonate particles (polychaetes, bryozoans, coral and sponges), 2) crypto-fauna that colonize the small holes and crevices (molluscs, crustaceans and polychaetes), 3) epifauna and endofauna, 4) eroding species including surface grazer, micro- and macro-borers (Sartoretto, 1996).

Mediterranean coralligenous surface is a substratum where sea urchins graze (e.g. *Sphaerechinus granularis*, or *Echinus melo*) that have (Laubier 1966) a different bioerosional activity on coralligenous concretions. Micro-borers include cyanobacteria (blue-green algae), green algae and fungi (Hong 1980). The group of macro-borers consist of mulluscs (e.g. *Lithophaga lithophaga*, *Gastrochaena dubia*) sipunculids, polychaetes and several excavating sponges (Sartoretto 1996). In particular *Cliona viridis* is one of the most abundant and powerful destructive sponge of calcareous substrata (Uriz et al. 1992, Rosell et al. 1999).

The biotic interactions are able to structuring coralligenous ecosystem. Their action is particular evident by the build-up of banks or rims by encrusting algae and invertebrate builders. Moreover, borers organisms can change, with their actions, the coralligenous frameworks.

In coralligenous habitat, animals and algae are submitted at trophic relations, to reduce the possibility to be preyed organisms may develop strategies to resistance. One of these strategies is to develop of skeletal structures that are made of carbonate or protein matters that confer at organism a protective layer for resist to the aggressions. In other animals, the defence is done by chemicals that confer they an unpalatable taste or even they result toxic (Martì 2002).

Most of organisms that supports the coralligenous habitat take the nutriment by pelagic system (suspension feeders or algae), and represent the organisms that constitute the higher living biomass (True 1970, Zabala and Ballesteros 1989). One example is the trophic relation between sea urchin, other invertebrates and encrusting algae (Ros 1978). The activity of the sea urchins is important for create free substrata where pioneer organisms can settle and grow, increasing the variability of organisms of the coralligenous assemblage.

The production of active substances, like chemicals utilised for defence or for inhibition of the territorial occupation of substrata, is a common strategy of several coralligenous organisms (Martì 2002). The main taxa that have these capacities are sponges, tunicates, bryozoans (Uriz et al. 1991). Less hospitable areas (low irradiance and small free substratum zone) are those in which organisms usually activate chemical defences.

1.1.3 Human activity

Over the last two centuries, the coralligenous assemblages were one of most affected and tapped marine habitat by human activities. Since long time people know that in this environment, there is a high richness of specie. At present day in fact, the Mediterranean coralligenous assemblage hosts numerous fishes, molluscs and crustaceans important for the fishing industry (Farrugio et al. 1993). The fishing activity induces an impoverishment and destruction of the habitats. Moreover, lost fishing gears could affect the assemblages covering the structures and modifying the hydro-dynamism (Bavestrello et al. 1997). Net and fishing line, under the effect of waves, could abrade the substratum and influence the sessile organisms. These persistent gears could host seasonal organisms that interfere on irradiance of substratum, uptake of nutrient and hydro-dynamism, changing the habitat condition. Finally, the degradation of plastic matter could release chemical substances (Mato at al. 2001) that may interfere on reproduction and growth of organisms.

Urbanization and global commerce of items have induce the built of artificial structures that modified permanently the natural habitat conditions. Often, this new habitat host, in addition at the endemic species, alien species common on the urban marine assemblage.

1.1.4 Global change

Increasing of temperature, acidification of seawater and increasing of storms episodes may threaten the coralligenous organisms. The impacts of climate change on marine habitat are already known (for example the mass mortality of gorgonians due at heat periods). Often, the changes happen quickly and not all organisms respond positively at the fast adaptation. The study of biological response after this change is necessary to understand how the modifications happen. Only with a good knowledge of all process is possible predict the changing in act. On recent years, the increasing of frequency of heat waves may affect with negative consequences the coralligenous species (epidemic disease and mass mortalities of organisms). This is particularly true, for sessile coralligenous organisms that are experiencing a fragmentation of population caused by mass mortality events (Coma et al. 2006, Calvo et al. 2011). The gap left by the species impacted is often resettled by aliens pecies, that compete for space, nutrient and resources.

Another threat for organisms that built calcareous structures is the acidification of seawater due at increasing of carbon dioxide on atmosphere. The coralline assemblages constructed by encrusting organisms are really affected by the increase of acidity. The effect that occurs is the dissolution of carbonates tissue and often of the thallus of algae. This effect is increased by periods of high temperature (Martin and Gattuso 2009). The trend of both factors until now considered show their alarming increasing. Finally, the intensification of storms (Beniston et al. 2007), resulted by the effect of global change, may contribute to erosion and destruction of habitat.

1.1.5 Pollution

Coralligenous assemblages can be affected by pollution, which tend to decrease their species diversity (Hong 1980,1982). In particular, bryozoans, echinoderms and crustaceans are the organisms most sensible, while molluscs and polychaetes were more resistant to pollution. Growth and calcification of coralline algae can be negatively affected by pollution (Littler 1976, Simkiss 1964). Bad land management, including deforestation, increased coastal sediments and the water turbidity in the coralligenous habitats (Airoldi 2003). Sediment may affect the hard bottom communities by covering sessile organisms and obstructing the filtering apparatus of suspension feeders (reducing

the growth and survivor rate) and limiting the algal photosynthesis production (Airoldi 2003, Irving and Connell 2002). Must consider the pollution resulted by the urban and industrial areas that directly discharge pollutants on rivers that flow to the sea. Increasing of organic substances and nutrients (from agriculture zones) influence positively the bloom of microalgae that affect the water turbidity, sedimentation and the frequency of mucilage events (Rinaldi et al. 1995). Furthermore, chemicals by industries may bring heavy metals that could compromise entire coastal zone, even if the latter are not bio-accumulate or bio-magnificate by marine organisms.

1.1.6 Species to preserve

For the considerations done until now, exist particular species that is well to preserve. The species considered are engineering species or species that have an essential role in the environment (for the nutriment, for nursery, for the simple protection of organisms).The loss of these species could induce a reduction of the ecosystem functions that could cause the collapse of coralligenous habitat, shifting the system from a higher complexity to a lower. Moreover human activities (harvesting, anchoring and scuba diving) can threat some organisms for example algae, suspension feeders (*Corallum rubrum*, gorgonians and bryozoans) (Weinberg 1991, Coma et al. 2004, Boudouresque et al. 1991, Linares and Doak 2010), molluscs (*Lithophaga lithophaga*, *Pinna nobilis*, *Pinna rudis*)(Boudouresque et al. 1991), and some target species of crustaceans and fishes that are fished (Mayol et al. 2000).

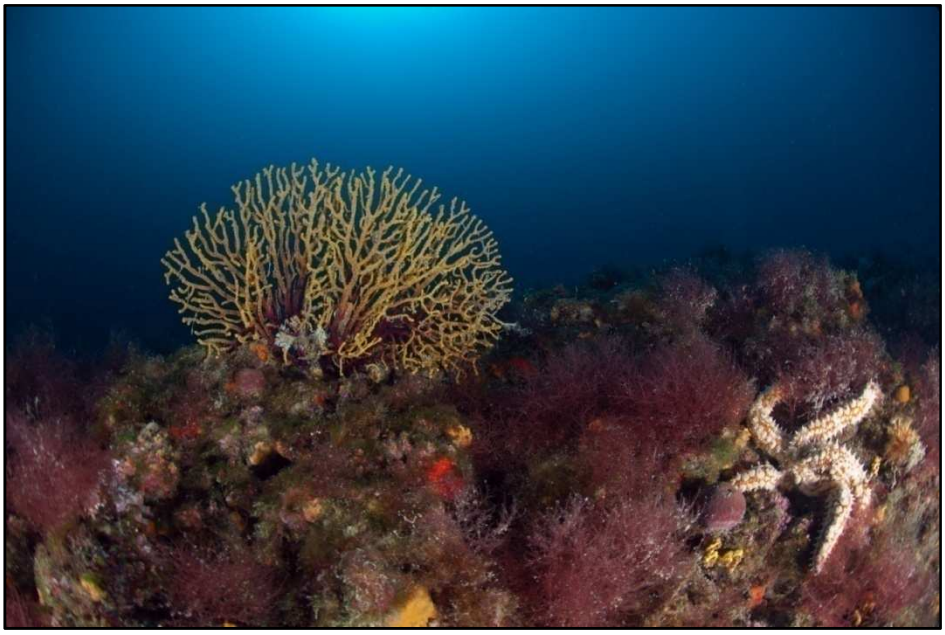

Figure 1 - Example of coralligenous community.

1.2 Gorgonians

Gorgonians are among the most ecologically important and spectacular organisms growing in the coralligenous habitats. They are suspension feeders or micro-predators that play a relevant trophic role in the assemblages (Weinberg 1976). At the best conditions the gorgonians growth in dense population that can influence the currents flows, as well as the intake of nutrients (Cocito et al. 2013). In fact, gorgonians attends in the nitrogenous and carbon marine cycle absorbing dissolved or particulate organic matter (DOM,POM), preying the smaller zooplankton component, and excreting metabolic waste and mucus (Coma and Gili 1994, Gili and Coma 1998, Wild et al. 2004). Most of gorgonians are able to create carbonate structures in the axis of their body. This skeleton can interpose at the maximum currents flows directions like obstacle, reducing the fluid velocity and creating turbulence in the surface layer that promote the acquisition of nutrients by polyps (Vögel 1981, Labarbera 1984). For this particular characteristic, they are considered as “habitat engineering” (Garrabou and Harmelin 2002, Coma et al. 2004, Linares et al. 2007).

1.3 The Mediterranean red gorgonian (*Paramuricea clavata*)

Kingdom	Animalia
Phylum	Cnidaria
Class	Anthozoa
Subclass	Octocorallia
Order	Alcyonacea
Suborder	Haloxonia
Family	Plexauridae
Genus	*Paramuricea*
Species	*Paramuricea clavata*(Risso, 1826)

Paramuricea clavata (Risso 1826), commonly named red gorgonian, is an endemic species of the Mediterranean Sea. The colonies have a red protein skeleton and carbonate spicules that provide elasticity and high resistance. The polyps are immersed in this matrix and they can retract if the conditions are not optimal (like high sedimentation or presence of mucilage). Dense populations can contribute at 40% of the biomass of benthonic assemblage (Gili 1986, True 1970). Although there are very few studies on its ecological role, *P.clavata* is considered a key species to maintain the complexity of the structure of the Mediterranean coralligenous community (True 1970, Ballesteros 2006).

1.3.1 Physical limits and populations distribution

Paramuricea clavata is widely distributed in the Mediterranean Sea between 10 to 100 meters in depth (Weinberg 1978, 1991). Although the its great diffusion, *P.clavata* presents patchy distributions (Linares 2007). Its feeding is facilitated by currents that convoy organic particulate and planktonic prey (Wainwright and Koehl 1976, Mariscal and Bigger 1977, Gili and Ballesteros 1991). Irradiance is another limiting physical factor. Weinberg (1976, 1978, 1991) suggests that the range of irradiance where were usually found the colonies of red gorgonians range between 0.12 and 27% of surface values (mean 7.6%). Temperature is another factor that may be considered for understands how the populations of red gorgonians are distributed; in particular, *P. clavata* seems very sensitive to high temperature for long periods (Cigliano and Gambi 2007, Coma et al. 2009, Fava et al. 2010, Previati et al. 2010). Results of studies, performed in laboratory or in nature, suggest that colonies of red gorgonian, at different

range of age, respond in a different way at the overheating of mean temperature. In adult specimens, high temperature conditions cause the reduced of their reproductive capacity (Linares et al. 2005, Cupido et al. 2008, 2009, Huete-Stauffer et al. 2011). Kipson et al (2012), in a laboratory experiment, showed the negative effect of the high temperature in the development of larval stage, where only a few larvae can survive until the metamorphic stage, moreover the results of metamorphosis are aberrant forms. Linares et al. (2008) found densities of *P. clavata* between 11 to 53 colonies m^{-2} that was not correlated by gradient of depth. In this study, the height of colony was also investigated (mean 24.2 ± 7.7 cm). The majority of colonies sampled, measured from 10 to 20 cm in height and there was a low percentage of small (<10 cm) or large (30-40 cm) colonies. In this study, a negative correlation between biomass and density of colonies was found, suggesting a possible intraspecific competition.

1.3.2 Growth rate and reproduction

Paramuricea clavata growth as tree form and it is considered a long-lived species that can persist in the habitat tens of years. Have not been established methodologies for detect the real age of the living colonies, but it is possible to estimate the age of organisms by growth rate of the main branch. Mistri and Ceccarelli (1994) measure that *P. clavata* could growth from 2.7 to 3.0 $cmyr^{-1}$ in height. Another recent study shows that the organisms can growth more slowly (approximately at 0.8 $cmyr^{-1}$ in height, Coma et al. 2001). According to these growth rates, there are colonies that should have much more than 50 years. However, it is always difficult to assign the correct age at the colonies because there are several interfering events: the main branch can be broken during the life of the gorgonian and it results in an underestimation of the real age, moreover mass mortality events can injure the colony that can reduce the growth rate. Velimirov (1976) observed that the colonies of gorgonians can growth faster thanks to high velocity current flow. This evidence was supported by studies that investigate the role of water movement in capturing prey and the respiration rate. Sebens (1984, 1987) shows that high currents and wave action could encourage the growth of coral colonies. At this condition, the respiration by oxygen diffusion is promoted as well as the metabolic costs. Finally, age of red gorgonian colonies can be obtained by a destructive method that is based on count the accretion circles in the basal cross section, each annual rings constituted by two different bands. The clear band is produced during the

fast development occurring in summer, the dark one is relative to the slow growth during the winter (Grigg 1974).

The red gorgonians present the colonies with separated sexes. The reproduction happens, at age from 7 to 13 yrs (Coma and Gili 1994), in asexual way, by fragmentation of parental colonies, or by sexual way, by the union of gametes. During the summer happens the output of gametes synchronous by the parental colonies. Eggs fertilized are retained in mucus on female colonies. Here happens the maturation until at larval stage. High currents could remove the embryos by mucous causing the premature larval death. The ciliate larvae are able to migrate, but not for high distance, therefore the settlement happens near the parental colonies. For this characteristic *Paramuricea clavata* appears with patchy distribution. Coma et al. (1995b) shows that the fecundity of red gorgonian decrease with increase of number of branches, this is explained by the difficult to access at food by polyps at different parts of branches. The bigger colonies contribute mostly in the production of gametes, but there are not data about their production in senescent period when have bigger branches.

1.3.3 Natural predation

Little is known on the natural predators of *Paramuricea clavata*. As other octocorals, red gorgonians are able to produce spicules, chemicals and secondary metabolites. These confer at the colonies unpalatable or anti-vegetative characteristic. Misti (2014), studying the organic content of gorgonians, affirm that there is energy available by predators on gorgonians tissue, but not all is easy use by consumers. Spicules (calcareous compounds produced by gorgonians for have high skeletal stiffness) and high mineralization of tissue play an important role on deterring predation of colonies. The coenenchyme cortex cropped is a little part of organism that is utilised for energetic needs by predators, the fate of a large part of organic tissue of gorgonian (and consequently the energy stored on it) is the decomposition after died. Moreover, is note that the spicules act as deterrent on predatory fish; this is confirmed by studies on predation of gorgonian. When the predator fishes were fed with pellet, which contain gorgonian spicules, their response was a clear reduction of food intake (Gerhart et al. 1988). Furthermore, chemical substances and metabolites may concur on the unpalatable taste on tropical gorgonians, beside to the important action that have against the spatial competitors (Van Alstyne and Paul 1992, Gerhart et al. 1988).

1.3.4 Paramuricea clavata mass mortality events

In the last decades, there have been some mass mortality events that have affected the distribution of red gorgonians, increasing the local extinctions, in the north-western Mediterranean Sea. The most impacted colonies were those living in the shallow waters, where the changes in physical conditions were more evident. The most important physical factors that guide the distribution of this species are the water temperature and the water column stability. The stratification of water column in late summer, with high temperature conditions in quite shallow waters are stress factors that could debilitate the red gorgonian colonies (Coma et al. 2009) and other suspension feeders. Not only the temperature take a part in the mortality events, other factors (like decrease of salinity caused by the rainfall of autumn seasons, the turbidity of the sea water and the presence of pathogenic bacteria) can also weaken the polyps colonies (Bavestrello et al. 1994). At these stress conditions, bacteria and other photogenes that are normally maintained under control by colony can became virulent (Cerrano et al. 2000). The damages that result, at begin of disease phases, is at the cortex level. The becoming brown and greyish of part of colonies, on contrasting the normal colour of healthy tissue, is an evident symptom of disease. After this phase, affected colonies show part of tissue that could secede, exhibiting the skeletal axes. Normally if this condition persists, the death of the entire colony is inevitable. Laboratory studies aimed to define the virulence capacity of bacteria show that only on stressful condition (normally with high temperature), the action of bacteria is relevant, and not all bacteria have the same necrotic effects (Martin et al. 2002). There are selected bacterial strains that are able to damage the colonies at high temperature. Bally and Garrabou (2007) confirmed the thesis that the effect of several selected virulent bacteria has a role on mass mortality events only at high temperature, when probably the defences of red gorgonians are low. Not all bacterial strains are virulent for the colonies of red gorgonians; there are several of them that normally live together the colonies and have a beneficial role in diseases prevention. Verzulli et al (2013) show that stressful conditions (anthropogenic activities, heat periods and other stress factors) may induce the expulsion of the symbiotic bacterial communities by red gorgonian, creating the opportunity of input of bacterial that are responsible of the disease. Other forms of damage of red gorgonians are the fishing activities (the anchorage, the losing the nets and the utilization of the fishing gear and fishing lines). The damage of fishing line is produced by the abrasive

action. The wounds are localised at the coenenchyme level and are produced by the rubbing of the fishing line. This effect increased by the attempts to escape of fishes from the hook. At last, attempts to escape could entangle other colonies that will have the same end. Like the line, the lost nets can cause at tens of colonies the same damage. With the action of the sea currents, the nets are able to destroy entire patches of coralligenous habitat. Moreover, when skeletal axes of red gorgonian are exposed could resettle epibionts whose expansions may form large aggregates (first hydroids than other competitors like bryozoans, serpulids and horny sponges) (Bavestrello et al. 1997). Massive presence of epibiontic may change the hydrodynamic flows on surrounding of colonies, reducing the feeding effect by polyps. Moreover, during high hydrodynamics condition, when the body of *Paramuricea clavata* is covered by epibionts, could increase the resistance at current flows till the break point. On naked branches, where is exposed the skeleton, the action of erosive polychaetes and nematodes could be too intense. The impairment of the structure of red gorgonian may cause the detachment of colonies by the substratum.

1.3.5 Recovery of *Paramuricea* patches

Mass mortality events could strongly threat the populations of Mediterranean red gorgonians. The effects of mortality episodes seem have an impact also several years after the event of death. The density of colonies can interact on the damage of mass mortality event. Is demonstrated that the mortality affect patch with low density of gorgonian colonies respect areas with high (Coma et al. 2006), and the size of gorgonian less affected are those < 15 cm. The recovery of tissues damaged is not possible for red gorgonian that present the skeletal axes exposed. Often, after several years of the mortality event, the colonies result intact, because the damaged axes may break, and the growth may continue with the live branch. The recovery of the population happens with the subsequent recruitment of juveniles produced by sexual reproduction. Is documented that, after a mass mortality events, the recruitment rate is low and it increase several years after that, when the population reach the reproductive size. Moreover, if the population is closed and if the mass mortality events highly reduced the number of colonies may happen an impairment of genetic variability of population that could threat it (Glascoigne and Lipcius 2004).

1.4 Study objectives

The global environmental change and human activity, including fishing, tourism, pollution and discharge of waste near the coastal zones, could threaten the coralligenous habitats (Verzulli et al 2013). Degradation of these habitats compromise their ecological functioning and services. Little is known of interaction between species that promote the construction of coralligenous framework. To preserve the marine coralligenous assemblage is important to identify the species responsible at its accretion, and understand the relation of these latter organisms with the living community. *Paramuricea clavata* may have a strong effect on structuring coralligenous benthic assemblages. The aims of the present study were to assess the ecological role of *Paramuricea clavata* in the coralligenous assemblages. For this purpose, benthic assemblages were studied in presence and absence of red gorgonians at two sites at the Tremiti islands.

2 Materials and methods

2.1 Study sites

The present study was carried on in two sites at Tremiti Islands (southern Adriatic Sea), which were declared Marine Protected Area (MPA) in 1989. The archipelago is located at the northern margin of the Apulian platform and is composed by six islands: San Domino, San Nicola, Cretaccio, Scoglio della vecchia, Caprara and Pianosa. Tremiti islands consist of discontinuous sequences of carbonate marine sediments, overlain by discontinuous continental deposit (Lollino and Pagliarulo 2008). The underwater orography of the islands consists on rising of slope that reaches about 80 m under sea level. The underwater slope presents an asymmetry of the inner of the extension of a continental shelf (Miccadei et al. 2012). In fact, the major slope is defined on the north-western coast, while the south-ester coast is characterize by seabed that slopes gently (Lollino and Pagliarulo 2008). The MPA is divided in three management zones with different protection levels (Figure 2). In the "A" zone the access is allowed only with the authority permission and only for scientific research or for guided tours. It is not allowed the sampling or the damage of both geological substrate and biological component. The commercial fishing is not allowed as well as is not permit the bathing, the not authorised diving and all the activity that can impact the environment. In the "B" zone are permitted the following activities: commercial and sportive fishing with stationary methods or trawling methods catch, diving and underwater photography. Is not permit every form of spear fishing and all the activities that could damage the biological component and the substratum, lastly it's not permit the transit of not authorized boat. In the "C" zone are permitted the authorised professional fishing and all type of sport fishing. According to preliminary observation two study sites with dense and quite shallow (less than 38 m) population of *Paramuricea clavata* were selected: "Secca di Punta Secca"("SPS"; N 42° 08' 25.5", E 15° 31' 31.4") and "Punto 55"("P55"; N 42° 06' 29.6", E 15° 31' 31.4") (Figure 3).

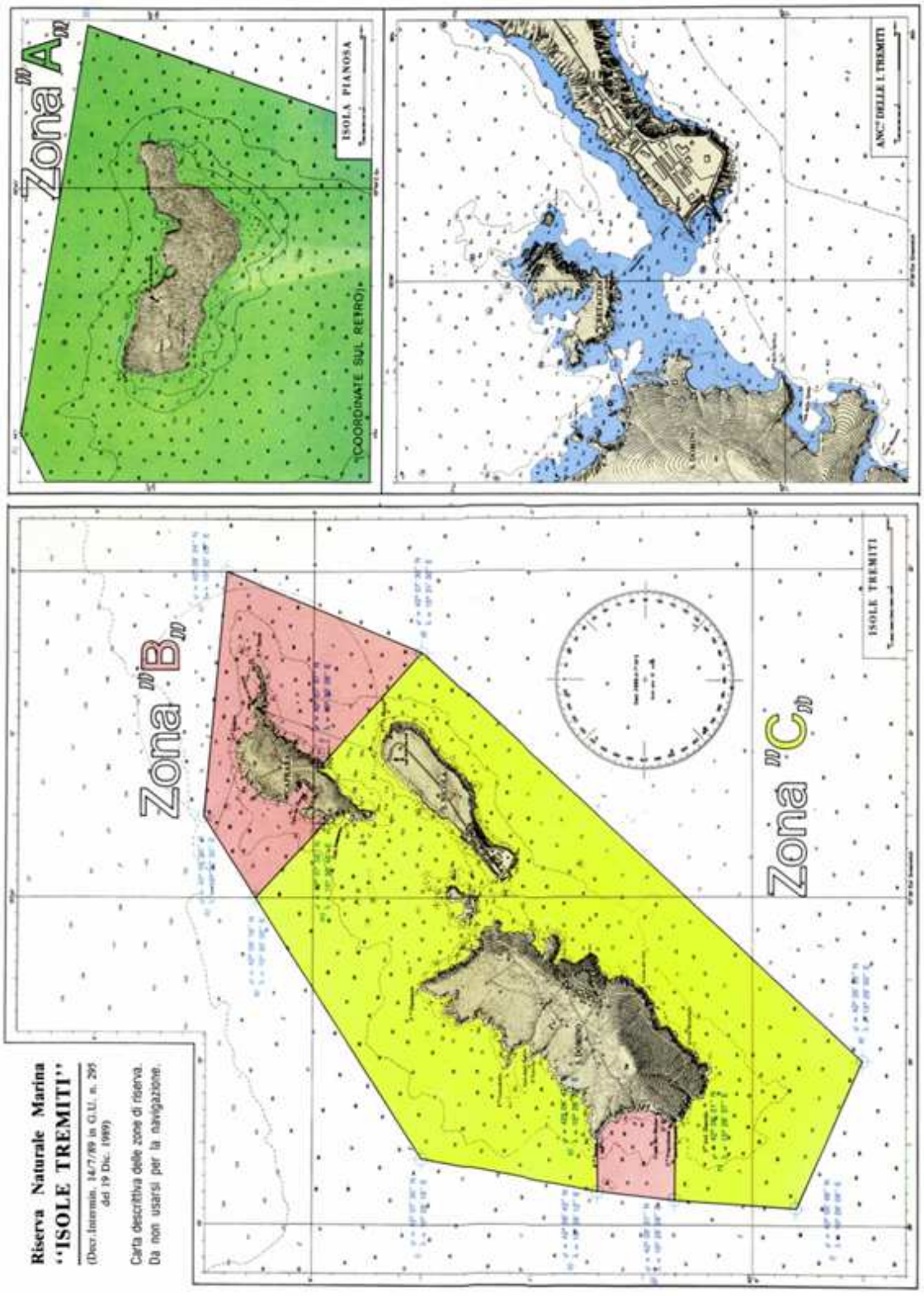

Figure 2 - Maps of archipelago of Tremiti Islands (green the "A" zone, pink the "B" zone and yellow the "C" zone).

Figure 3- Tremiti Islands maps. Is evidenced the two sampling point: "Secca di Punta Secca" ("SPS") and "Punto 55" ("P55").

2.2 Sampling design

The null hypothesis of this thesis was that *Paramuricea clavata* (Risso, 1826) at high density (forming a sort of "forests") has no effects on benthic organisms that live in the coralligenous assemblage. The sampling design (Figure 4) considered three orthogonal fixed factors: "Site" with two levels ("SPS" for "Secca di Punta Secca" and "P55" for "Punto 55"),the presence of gorgonian "forests" with two fixed levels (the presence ,Y, and absence ,N), and plots randomly replicated five times for each site and gorgonian forest combinations.

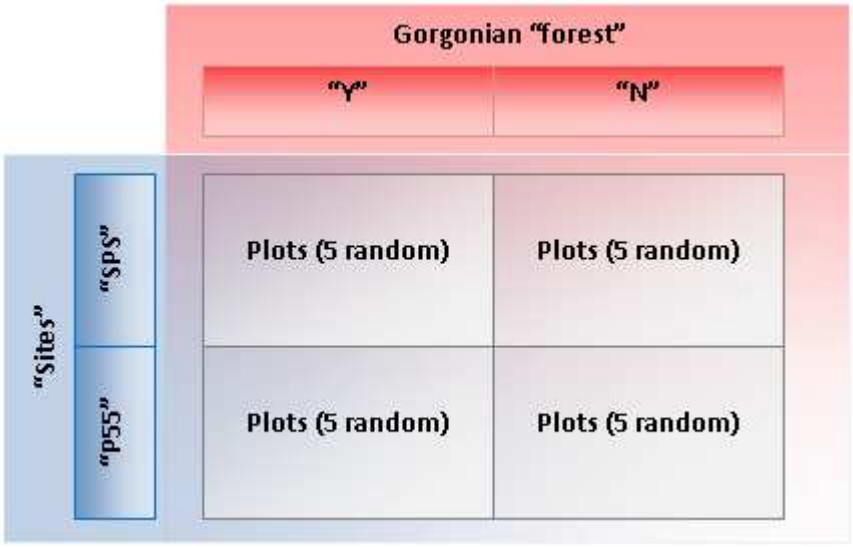

Figure 4 - Schematic representation of sampling design. With red color the factor Gorgonian "forest" (presence "Y", absence "N"), with blue color the factor "Site" (Secca di Punta Secca "SPS", Punto 55 "P55"). With light purple color the factor plots nested on Site (with five levels).

2.3 Sampling methods

The study was based on photographic samples, carried out by digital cameras (Olympus xz-1 and Cannon G11). The light sources were provided by two flashes INON D-2000. To delimit the areas of work has been used two different squares. The evaluation of gorgonian abundances and habitat complexities was analysed in plots of 50 × 50 cm (Paravicini et al. 2010), while the percent covers of epibenthic organisms were estimated within the plots by 21 × 28 cm quadrates.

2.4 Vertical complexity index

Habitat complexity induced by gorgonians and other vertically developed species were assessed by a vertical complexity index (*VCI*). It was obtained measuring the contour shape of organisms in pictures orthogonal to the substratum and dividing by the base of the pictures (i.e. 50 cm). All the measures were obtained by the ImageJ® software.

2.5 Percent covers estimations

All percent covers estimations were carried out on a 400 cells grid superimposed to the pictures and using the Photoquad® software (Trygonis and Sini, 2012). Organisms were identified to the lowest possible taxonomic level.

2.6 Diversity indices

For each photographic benthic assemblages sample the species richness (*S*), the Pielou's evenness index (*J'*) and the Shannon's diversity index (*H'*, based on log_2) were calculated.

2.7 Statistical analysis

Differences in assemblage structures were represented using principal coordinate analysis (PCO unconstrained ordination plot; Gower 1966) based on Bray-Curtis similarities of square root-transformed percent cover data. Differences in community structures according to the sampling design were assessed by permutational multivariate analysis of variance (PERMANOVA, $\alpha = 0.05$; Anderson 2003). When less than 999 unique values in the permutation distribution were available, asymptotical Monte Carlo *P*-values were used, instead of permutational *P*-values. Multivariate tests were performed on Bray-Curtis similarities of square root-transformed percent cover data. Significant interactions among main factors were investigated by post-hoc pair-wise tests.

Differences in gorgonian abundances, *VCI*, community structures, species abundances and species diversity according to the sampling design were assessed by analysis of variance (ANOVA, $\alpha = 0.05$) following the general principles applied in ecology (Underwood, 1997). Homogeneities of variances were tested by the Cochran C test and data transformations were applied when necessary. In case of light homogeneity of variances (Cochran C test $P<0.05$), $\alpha = 0.01$was applied. Significant interactions among main factors were investigated by post-hoc SNK tests.

Univariate analyses were performed by R® software, while multivariate analyses and diversity indices calculation were obtained using Primer®.

3 Results

3.1 Gorgonian abundance and habitat complexity

The gorgonians in the randomly selected "forested" plots showed similar abundances at the two study sites (percent cover 43%, Figure 5) and are higher than in the non-forested plots, as confirmed by the statistical test (Table 1). As could be expected, the habitat complexity, measured by the vertical complexity index (*VCI*) were significant higher in the gorgonian "forest" plots, while there was no significant difference between sites (Figure 5 and Table 2).

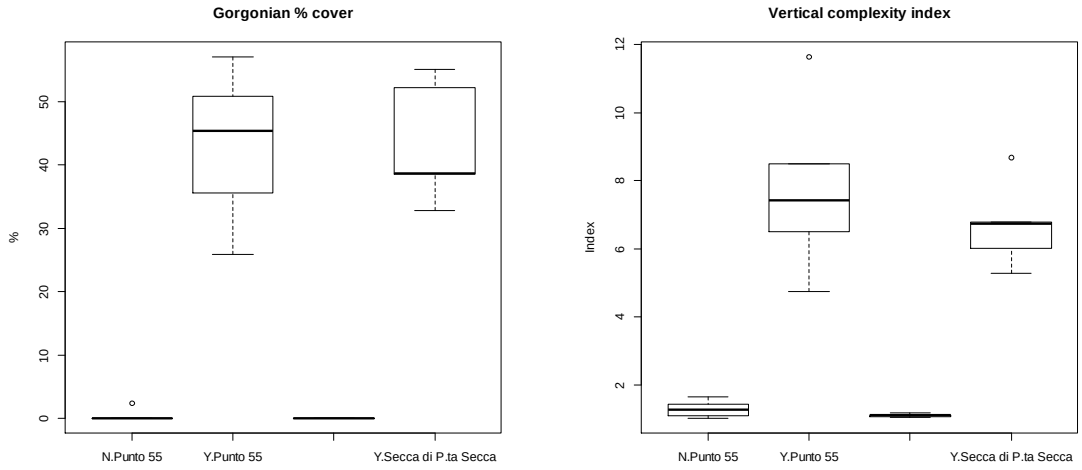

Figure 5 - Box plot of gorgonian percent cover (left) and vertical complexity index (right) in the selected plots in the two sites (SPS – "Secca di Punta Secca", P55 – "Punto 55") with and without *Paramuricea clavata* (N, Y).

Table 1 – ANOVA table on *Paramuricea clavata* percent cover (Cochran's C test *P*> 0.05).

Source	df	SS	MS	F	*P*	
Site	1	0.0	0.0	0.0000	1.0000	
Forest	1	9236.4	9236.4	149.0451	1.60E-09	***
Site×Forest	1	1.2	1.2	0.0186	0.8933	
Residual	16	991.5	62			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 2 –ANOVA table on cuberoot transformed *VCI* data (Cochran's C test *P*<0.05).

Source	df	SS	MS	F	*P*	
Site	1	0.0224	0.0224	1.3698	0.259	
Forest	1	3.6853	3.6853	225.2910	7.57E-11	***
Site×Forest	1	0.0011	0.0011	0.0662	0.8003	
Residual	16	0.2617	0.0164			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

3.2 Possible effects of gorgonian forests on sessile benthic assemblages

3.2.1 Effects on single taxa

Overall, 33 sessile taxa belonging to algae (11), sponges (9), cnidarians (4), polychaetes (1), bivalve molluscs (1), crustaceans (1), bryozoans (3), tunicates (3), were found. The possible effects of gorgonian forests on the abundance of each single taxon are summarised in (Table 3).

Several species showed distribution pattern related to the sites, but some of them seem affected by the gorgonian presence, at least in one site. In particular Cladophorales, green filamentous algae, showed both local variability, among plots, and a significant interaction Forest × Site (Figure 6a and Table 4), being more abundant in the P55 site in presence of gorgonians compared to in P55 without gorgonians (SNK test $P<0.01$) and to SPS with gorgonians (SNK test $P<0.01$). Conversely, the green algae *Codium coralloides*, which also showed both local variability and a significant interaction Forest × Site (Figure 6b and Table 5) was more abundant in the SPS site in presence of gorgonians compared to in SPS without gorgonians (SNK test $P<0.01$) and to P55 with gorgonians (SNK test $P<0.001$).

Algal turf (Figure 6c and Table 6) was more abundant in SPS, compared to P55 both in presence and absence of gorgonians (SNK tests $P<0.001$), and in this site was negatively affected by the presence of gorgonians (SNK test $P<0.001$).

The filamentous red algae *Sphaerococcus coronopifolius*(Figure 6d and Table 7) was very rare in SPS and negatively affected by the gorgonians in P55 (SNK test $P<0.001$).

The abundance of encrusting coralline red algae (ECR; Figure 6e) was significantly promoted by the presence of gorgonians in both sites and with comparable values (Table 8).

The encrusting red sponges (Figure 6f, Table 9) were nearly absent in SPS, while significantly promoted by the presence of gorgonians in P55 site (SNK test $P<0.001$).

Table 3 – Summary of ANOVA tests on the abundances of single taxa found. When Plot (Site × Forest) was not significant (*P*>0.25) it was eliminated.

Taxon	Transf.	Cochran's C test	Plot (Site × Forest)	Site × Forest	Forest	Site
Algal turf	None	Ns	Ns	*	***	***
Mucilaginous algae	^1/3	Ns	**	ns	ns	*
ECR	None	Ns	**	ns	*	ns
Fleshy red algae	^1/4	Ns	Ns	ns	ns	***
Peyssonnelia spp.	^1/2	Ns	Ns	ns	ns	ns
Sphaerococcus coronopifollus	^1/3	Ns	**	*	ns	**
Wrangelia penicillata	^1/4	*	Ns	ns	ns	ns
Dictyotales		***				
Cladophorales	None	Ns	*	*	*	ns
Codium coralloides	^1/2	Ns	***	*	*	**
Halimeda tuna	^1/2	Ns	Ns	ns	ns	***
Dark massive sponges		***				
Encrusting Red Sponges	^1/4	Ns	Ns	**	**	**
Ind.sponges		***				
Agelas oroides	^1/4	Ns	Ns	ns	ns	***
Axinella spp.		***				
Chondrosia reniformis		***				
Dysidea spp.		***				
Ircinia variabilis		***				
Tedania anhelans		***				
Balanophyllia sp		***				
Cribrinopsis crassa		***				
Eunicella cavolini		***				
Parazoanthus axinellae	^1/2	Ns	eliminate	ns	ns	***
Serpulids	^1/2	Ns	Ns	ns	ns	ns
Rocellaria dubia		***				
Barnacles		***				
Encrusting bryozoans	none	ns	Ns	ns	ns	ns
Myriapora truncate	^1/2	ns	Ns	ns	ns	***
Pentapora fascialis	^1/3	ns	Ns	ns	ns	ns
Colonial ascidians		***				
Halocynthia papillosa	^1/3	*	Ns	ns	ns	ns
Styela plicata		***				

Signif. codes: <0.001 '***' <0.01 '**' <0.05 '*'>0.05 'ns'

If Cochran's C test was significant (*P*<0.05) then codes: <0.001 '**' <0.01 '*' <0.05 'ns'

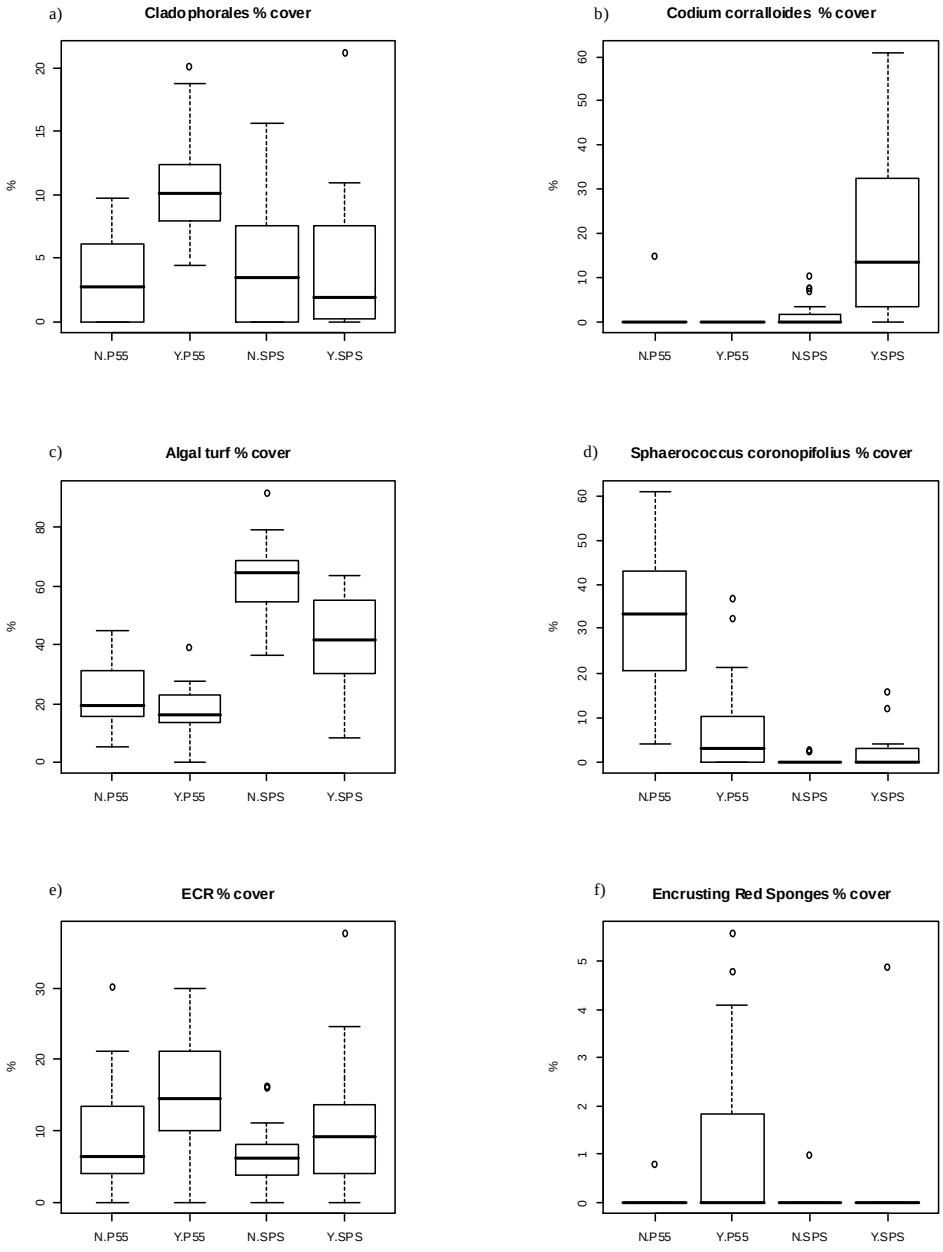

Figure 6- Box plot of percent cover of selected taxa in relation to the presence of the gorgonian "forest" (Y, N)and site (P55 – "Punto 55" and SPS – "Secca di Punta Secca").

Table 4 - ANOVA table on Cladophorales data (Cochran's C test *P*>0.05).

Source	Df	SS	MS	F	*P*	
Site	1	112.81	112.812	3.324	0.0870	
Forest	1	221.11	221.113	6.514	0.0213	*
Site × Forest	1	284.26	284.258	8.375	0.0106	*
Plot(Site × Forest)	16	543.07	33.942	1.866	0.0426	*
Residual	60	1091.43	18.190			

Signif. codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 5 - ANOVA table on square root transformed *Codium coralloides* data (Cochran's test *P*> 0.05).

Source	df	SS	MS	F	*P*	
Site	1	82.647	82.647	12.855	0.0025	**
Forest	1	37.375	37.375	5.814	0.0283	*
Site × Forest	1	48.714	48.714	7.577	0.0142	*
Plot(Site × Forest)	16	102.864	6.429	7.257	0.0000	***
Residual	60	53.157	0.886			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 6 - ANOVA table on algal turf data (Cochran's C test *P*>0.05).

Source	df	SS	MS	F	*P*	
Site	1	20743.2	20743.2	94.393	0.0000	***
Forest	1	3914.4	3914.4	17.813	0.0007	***
Site × Forest	1	1468.9	1468.9	6.684	0.0199	*
Plot(Site × Forest)	16	3516.1	219.8	1.680	0.0760	
Residual	60	7848.4	130.8			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 7 - ANOVA table on cube root transformed *Sphaerococcus coronopifolius* data (Cochran's test *P*> 0.05).

Source	df	SS	MS	F	P	
Site	1	64.288	64.288	35.924	0.0000	***
Forest	1	9.938	9.938	5.553	0.0315	*
Site × Forest	1	21.602	21.602	12.071	0.0031	**
Plot(Site × Forest)	16	28.633	1.790	4.042	0.0000	***
Residual	60	26.563	0.443			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 8 - ANOVA table on ECR data (Cochran's C test *P*>0.05).

Source	df	SS	MS	F	*P*	
Site	1	270.85	270.85	2.630	0.1244	
Forest	1	537.68	537.68	5.220	0.0363	*
Site × Forest	1	35.64	35.64	0.346	0.5646	
Plot(Site × Forest)	16	1647.95	103.00	2.417	0.0072	**
Residual	60	2556.95	42.62			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 9 - ANOVA table on fourth root transformed encrusting red sponges data (Cochran's test *P*> 0.05).

Source	df	SS	MS	F	P	
Site	1	1.157	1.157	10.655	0.0049	**
Forest	1	1.433	1.432	13.191	0.0022	**
Site × Forest	1	1.183	1.183	10.896	0.0045	**
Plot(Site × Forest)	16	1.738	0.109	0.6456	0.8333	
Residual	60	10.092	0.168			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

3.2.2 Effects on assemblages structures

Similarities among assemblages found in the two study sites and in relation to the presence and absence of the gorgonian forest are reported in Figure 7. The plot clearly shows the separations between assemblages belong to the two different sites, but also some degree of separation between assemblages occurred in presence and absence of gorgonians. These observations are confirmed by the PERMANOVA test (Table 10), which highlight a significant local variability, among plots, and a significant interaction effect of Forest × Site. Difference in the structures of benthic assemblages between sites with and without gorgonians was confirmed by the pair-wise tests (Table 11 and Table 12) and the effect of the gorgonians seem stronger in P55 site.

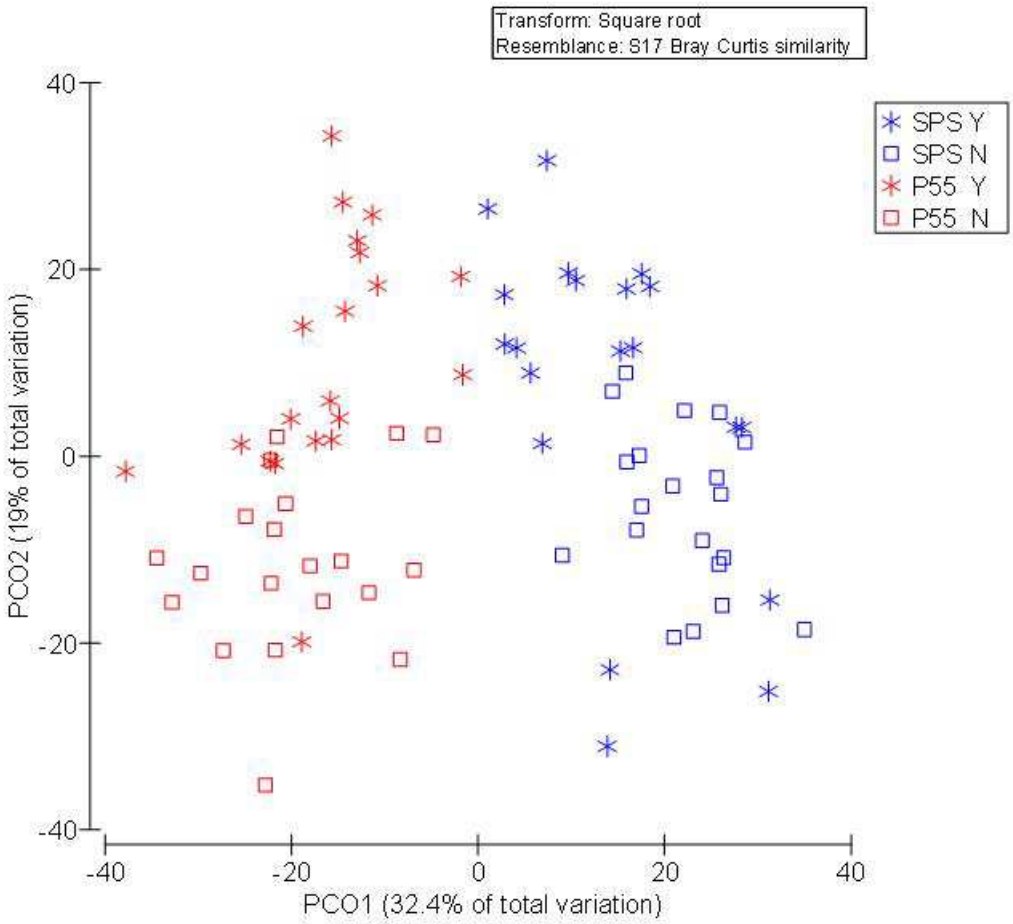

Figure 7 – PCO ordination plot showing similarities among assemblages found in the two site "Secca di Punta Secca" (SPS) and "Punto 55"(P55) in presence (Y) and absence (N) of *Paramuricea clavata* forests.

Table 10 – PERMANOVA tests.

Source	df	SS	MS	Pseudo-F	*P*(perm)	Unique perms
Forest	1	6934.4	6934.4	5.1011	0.0033	9940
Site	1	26196.0	26196.0	19.2700	0.0001	9937
Forest × Site	1	5549.7	5549.7	4.0825	0.0015	9939
Plot(Site × Forest)	16	21750.0	1359.4	2.2389	0.0001	9794
Res	60	36429.0	607.2			
Total	79	96859.0				

Table 11 – Pair-wise tests between sites in presence and absence of gorgonians.

Pair-wise tests (Y)				
Groups	**T**	**P(perm)**	**Unique perms**	**P(MC)**
PS, P55	2.6667	0.0085	126	0.0003
Average Similarity between/within groups				
	PS	**P55**		
SPS	60.731			
P55	47.885	61.091		
Pair-wise tests (N)				
Groups	**T**	**P(perm)**	**Unique perms**	**P(MC)**
PS, P55	4.4924	0.0083	126	0.0001
Average Similarity between/within groups				
	PS	**P55**		
PS	66.71			
P55	43.872	61.044		

Table 12– Pair-wise tests between presence and absence of gorgonians in the two study sites.

Pair-wise tests (SPS)				
Groups	**T**	**P(perm)**	**Unique perms**	**P(MC)**
Y,N	1.8516	0.0584	126	0.015
Average Similarity between/within groups				
	Y	**N**		
Y	60.731			
N	57.474	66.71		
Pair-wise tests (P55)				
Groups	**T**	**P(perm)**	**Unique perms**	**P(MC)**
Y,N	2.5164	0.0073	126	0.0002
Average Similarity between/within groups				
	Y	**N**		
Y	61.091			
N	54.271	61.044		

3.2.3 Effects on species diversity

Selected species diversity indices are reported below (Figure 8). Overall, species richness, evenness and species heterogeneity was significantly higher in P55 than in SPS, moreover a significant effect of the gorgonian forests in increasing species diversity was observed and statistically confirmed for all of the indices (Table 13, Table 14, Table 15).

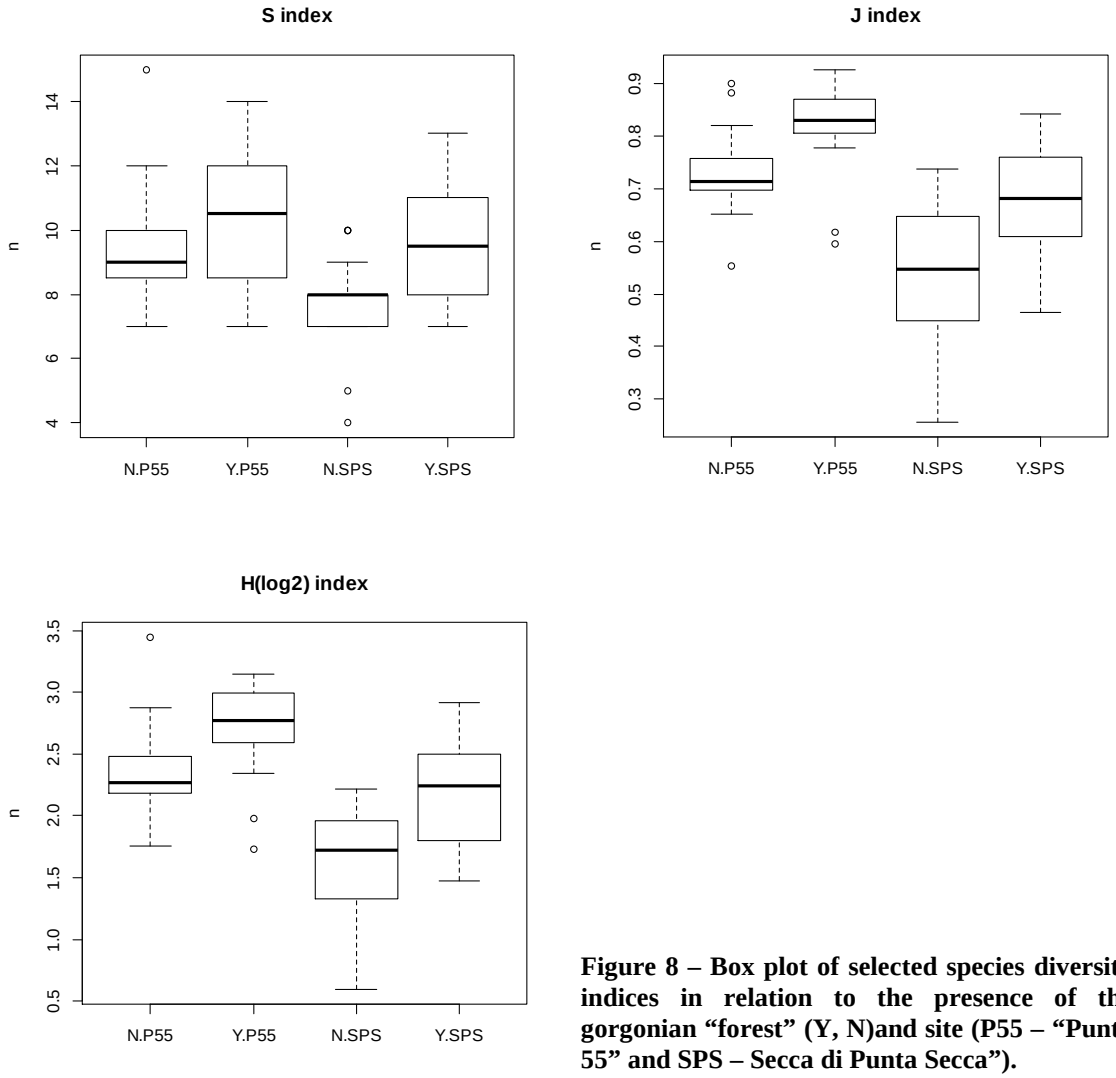

Figure 8 – Box plot of selected species diversity indices in relation to the presence of the gorgonian "forest" (Y, N)and site (P55 – "Punto 55" and SPS – Secca di Punta Secca").

Table 13 – ANOVA table on species richness (*S*) data (Cochran's C test *P*>0.05).

Source	df	SS	MS	F	P	
Site	1	27.613	27.613	8.320	0.0108	*
Forest	1	30.012	30.013	9.043	0.0084	**
Site × Forest	1	5.512	5.513	1.661	0.2158	
Plot(Site × Forest)	16	53.100	3.319	1.075	0.3980	
Residual	60	185.250	3.088			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 14 - ANOVA table on evenness (*J'*) data (Cochran's C test *P*>0.05).

Source	df	SS	MS	F	P	
Site	1	0.546	0.546	46.839	0.0000	***
Forest	1	0.257	0.257	22.059	0.0002	***
Site × Forest	1	0.012	0.012	1.057	0.3192	
Plot(Site × Forest)	16	0.187	0.012	1.298	0.2286	
Residual	60	0.539	0.009			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

Table 15- ANOVA table on Shannon index (*H'*) data (Cochran's C test *P*>0.05).

Source	df	SS	MS	F	P	
Site	1	8.159	8.159	40.924	0.0000	***
Forest	1	4.539	4.539	22.768	0.0002	***
Site × Forest	1	0.275	0.275	1.381	0.2572	
Plot(Site × Forest)	16	3.190	0.199	1.351	0.1983	
Residual	60	8.857	0.148			

Signif.codes: <0.001 '***' <0.01 '**' <0.05 '*'

4 Discussions

Benthic communities are very variable at local scale (Fraschetti et al. 2005, Piazzi et al. 2004) and the comprehension of their distribution is extremely important for any conservation policies. In particular, gorgonians increase the three-dimensional structure of the habitat and may affect the other sessile and vagile organism by a wide range of possible interaction (Scinto et al., 2009; Ponti et al., 2011; Ponti et al., 2012; Ponti et al., 2014). The loss of this kind of "animal forests" is advocated as one of the most important causes of over-simplification and threat of the worldwide benthic ecosystems (Rossi, 2013). Understanding the role of gorgonians on benthic community of rocky substrata is important in order to establish the complex network of relationship between organisms. The gorgonians, for their nature, have a patchy distribution and their occurrence may affect the whole benthic assemblage. At the Tremiti Islands, dense populations of *Paramuricea clavata* are very rare, often quite deep, and in the present study two of the shallowest populations were considered. The selected sites are located at different geographical zone of the archipelago. "Secca di Punta Secca" (SPS), which is located northern to the north-est tip of Caprara island, is exposed to the prevailing south-west currents. "Punto 55" (P55), being partially sheltered by San Domino island (approximately six hundred meters of distance from the coastline), is less influenced by the prevailing currents, but occasionally this site is affected by the less intense northern currents. Moreover, the two sites differ by their geomorphologies: while SPS is a submerged prolongation of the side of the island, the peak of P55 is an isolated outcrop.

Although in different place, characterised by different environmental conditions, the two investigated gorgonian populations had similar densities, in terms of percent cover, and showed the same habitat complexity, as measured by the vertical complexity index (*VCI*).

Overall, the benthic assemblages found in the two sites were significantly different each other, never the less in both places, clear differences between assemblages associated to the gorgonian forest sand those present in the surrounding non-forested areas were found. According to the similarity analysis, although with different intensities, the presence of dense populations of red gorgonians appears responsible of a shift of the benthic assemblages in the same direction, which also seems reduce the differences

between sites. This effect can be due to more sheltered and stable conditions offered by the gorgonian forests, compared to the exposed surrounding areas.

The presence of *Paramuricea clavata* affects the abundance of six taxa among the thirty-three found by the photo-sampling. Encrusting coralline red algae were significantly more abundant in the gorgonian under stories at both sites. This result indicates that the gorgonians may promote the development of these calcareous algae, which are the main builders of the coralligenous habitats. Other detected possible gorgonians effects were site specific. For instance, gorgonians seemed to promote the green algae *Codium coralloides* at PSP as well as the Cladophorales green algae and the encrusting red sponges at P55. Conversely, the brushy red algae *Sphaerococcus coronopifolius* were more abundant at P55 in absence of the gorgonians. Notably, algal turf seems to be lowered by the gorgonians "forest", especially at SPS, where it was more abundant on overall. Basically, in absence of gorgonians some filamentous and brushy algae tend to dominate the benthic assemblages at the expense of calcareous algae and invertebrates.

In both study sites, red gorgonian “forests” seem to have a positive effect on species diversity increasing species richness and evenness, and therefore the overall species heterogeneity. These animal “forests” may promote micro-habitat heterogeneity and complexity that can increase the opportunities for settlement and favourable growing conditions for several species. While filamentous and brushy algae tend to homogenizethe habitat.

Differences in assemblages structures between sites could be related to the local larval supply, water quality, sedimentation and hydrodynamic conditions that may vary between sites. Gorgonians effects may be related to both biological and physical factors. Being passive filter feeders (they cannot create currents from their own) and active predators (polyps capture preys by nematocysts) they can intercept and feed on settling larvae before sinking and settling. Moreover, they may compete for food with other filter-feeders and/or for space, producing allelochemicals. On the other hand, gorgonians could affect settlement and recruitment processes by modifying microscale hydrodynamic conditions, sedimentation rates by limiting resuspension processes, and algal development by creating shading effects that may reduce the photosynthetic activity. In the studied sites, the presence of gorgonians mainly controls the growth of erected algae in favour of encrusting algae and some sessile invertebrates. Therefore, depending on the trophic conditions and turbidity of the water, the gorgonian rarefaction

may favour a more or less evident shift toward more autotrophic communities and a trivialization of coralligenous assemblages.

In a globally changing world, increasingly subject to natural and anthropogenic stresses, understand the ecological role of red gorgonians in the Mediterranean coralligenous habitats is essential in order to design appropriate monitoring programs and conservation policies, especially in the marine protected areas.

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